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Evaluation of the Copan eSwab[®], a liquid-based microbiology transport system, for the preservation of *Neisseria gonorrhoeae* at different temperatures

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Aims and objectives: To evaluate the survival duration of *Neisseria gonorrhoeae* from male urethral discharge specimens collected using the Copan eSwab[®] liquid-based microbiology transport system, at both ambient and refrigerator temperatures.

Methods: Three urethral swabs (one Dacron, two Copan eSwabs[®]) were collected from each male patient presenting with purulent urethral discharge to a community-based primary healthcare centre in Johannesburg. The Dacron swab was directly inoculated onto New York city agar medium, and the Copan eSwabs[®] transported and held at room and refrigerator temperature, for daily sub-culture onto New York city agar over a total period of seven days (168 h). The utility of Copan eSwabs[®] for the transport and survival of *N. gonorrhoeae* at different temperatures was determined by comparison to culture obtained by 'gold standard' direct plate inoculation.

Results: *N. gonorrhoeae* isolation rates from Copan eSwabs[®] at fridge temperature and ambient temperature were as follows: 87.9% vs 79.3% at 48 h; 67.2% vs 60.3% at 72 h; 60.3% vs 22.4% at 96 h; and, 53.4% vs 3.4% at 120 h, respectively. The viability of subculture decreased significantly from eSwabs[®] maintained at room temperature from 96 h onwards of specimen collection.

Conclusion: To ensure the preservation and an acceptable isolation rate of *N. gonorrhoeae* from urethral discharge specimens, Copan eSwabs[®] should be transported and maintained at refrigerator temperatures, and must reach the processing laboratory by at least 120 h (5 days) after collection.

Keywords: amies transport medium, Copan eSwab, *Neisseria gonorrhoeae*

Introduction

The probability of success in the isolation of pathogenic *Neisseria* species from clinical specimens is related to three factors, namely: the amount of care taken in obtaining good specimens and inoculating them correctly; the provision of a culture medium capable of growing demanding strains of *Neisseria* from small inocula; and, the inclusion of selective agents in the medium which are capable of preventing overgrowth of commensal organisms, but do not inhibit the growth of the species required. Cultures from clinical specimens often lose viability and become contaminated during shipment to the laboratory. Thus, specimen collection and transport are vital steps in the pre-analytical phase, as downstream reading, interpretation and reporting by technical staff are dependent on specimen quality and organism survival while in transit to the processing laboratory. Amies transport medium with or without charcoal has been used extensively to transport *Neisseria gonorrhoeae* and may preserve viability for up to 48 h.¹ More recently, liquid-based microbiology is becoming increasingly utilised to enhance the efficiency of specimen processing and maximise recovery of micro-organisms.² Specimen collection devices transform samples into liquid to improve specimen release and elution, and allow for automation where needed. The advantages of this are that a homogeneous suspension is obtained, which provides multiple sample aliquots for standardisation of the diagnostic process and enhances the preservation micro-organisms. The Copan liquid Amies elution swab (eSwab)[®] is an FDA-approved multipurpose specimen collection and transport device, consisting of a unique nylon fibre flocked swab and 1 ml Amies transport medium contained in a polypropylene screw-cap tube.³ It maintains the viability of aerobes, anaerobes and fastidious bacteria, and preserves nucleic acids. According to the manufacturers, Copan eSwabs[®]

preserve *N. gonorrhoeae* at ambient temperature and fridge temperature for up to 24 h from time of collection.³

N. gonorrhoeae is the predominant cause of male urethritis syndrome in South Africa.⁴ Establishing an optimal collection and transport system for urethral discharge specimens obtained from peripheral sentinel surveillance sites within South Africa is essential for maximal isolation and recovery of the organism. In view of increasing rates of gonococcal resistance worldwide,⁵ and in accordance with the World Health Organization Global Antimicrobial Resistance Surveillance System (GLASS) recommendations,⁶ it is essential for countries to monitor *N. gonorrhoeae* susceptibility profiles as a core component of antimicrobial resistance surveillance.

The Centre for HIV and STIs (CHIVSTI) of the National Institute for Communicable Diseases (NICD), based in Johannesburg, South Africa, co-ordinates the aetiological surveillance of STI syndrome cases as part of the NICD GERMS-SA surveillance program. This includes antimicrobial resistance testing of *N. gonorrhoeae* isolates from male urethritis specimens. Surveillance sites are primary healthcare facilities (PHCs) situated in each of the nine provinces of South Africa. For the 2015–2016 surveillance period, PHCs were located at distances of 191 km (North West province) to 1 070 km (Eastern Cape province) from CHIVSTI. Road transport of specimens incurred transit times of, on average, three days (range: 1 – 6 days).

The aim of this study was to evaluate the utility of Copan eSwab[®] for the transport and preservation of *N. gonorrhoeae* from urethral discharge specimens, and to estimate the duration of organism survival at both ambient and fridge temperatures.

Study objectives

- To evaluate the utility of Copan eSwabs® as a transport and holding medium for *N. gonorrhoeae* by comparing culture yields to the gold standard direct plate inoculation.
- To estimate the duration of survival of *N. gonorrhoeae* in Copan eSwabs® at ambient and refrigerator temperatures

Materials and methods

Specimen collection and transport

Consecutive consenting male patients presenting with urethral discharge syndrome to Alexandra Health Centre, Johannesburg, were recruited for routine aetiological surveillance, in accordance with the NICD GERMS-SA STI surveillance protocol. Routine specimen collection included a urethral Dacron swab inoculated directly onto New York city (NYC) agar (Diagnostic Media Products, NHL, South Africa). This was placed in a holding candle jar. Two additional endourethral specimens per patient were collected using Copan eSwabs® (Copan Italia Spa, Italy); these were placed in accompanying Amies transport media for transport on ice and at ambient temperature, respectively. All specimens reached the processing laboratory (STI reference laboratory, NICD, Johannesburg, South Africa) on the day of collection.

The order of specimen collection entailed that the first specimen from every patient was collected using a Dacron swab for direct plate inoculation. For even-numbered enrolled patients, the second and third eSwabs were held at fridge and room temperatures, respectively; whereas, this order was reversed for odd-numbered enrolled patients.

Laboratory processing

On arrival at the STI laboratory, the NYC agar plate was incubated in CO₂ at 35–37°C and examined for growth daily for a total of three days.

The Copan eSwabs® were transported and held at room and refrigerator temperature, for daily sub-culture onto NYC agar over a total period of seven days.

For sub-culture, 100 µl Amies media from each of the two eSwabs® were inoculated onto one half of a NYC agar plate, which was labeled appropriately to reflect the two temperatures at which the swabs had been maintained. The plate was incubated in CO₂ at 35–37°C and examined daily for a total of three days.

Culture colonies growing on NYC agar were identified as *N. gonorrhoeae* using phenotypic methods: Gram stain, oxidase test and the Phadebact® Monoclonal GC test (MKL Diagnostics AB, Sollentuna, Stockholm, Sweden).

Data entry and analysis

Microbiological data for each patient sampled were documented on laboratory working cards. This included information on whether *N. gonorrhoeae* was cultured from direct plate inoculation; as well as whether isolates were obtained on sub-culture of eSwabs® held at room and refrigerator temperatures at 24 h intervals from time of collection. Results were captured on a Microsoft Excel spreadsheet for statistical analysis.

The proportion (%) of *N. gonorrhoeae* isolates cultured from eSwabs® held at ambient and refrigerator temperatures at 24 h of collection, compared to isolation rates following gold-standard direct plate inoculation, was determined with Wilson's 95% confidence intervals around the estimate.

The viability of *N. gonorrhoeae* sub-culture at 24, 48, 72, 96, 120, 144 and 168 h following collection was determined for Copan eSwabs stored at both ambient and fridge temperatures by calculating proportions of isolates from sub-cultures as a fraction of those expected to yield *N. gonorrhoeae*, also with Wilson's 95% confidence intervals around the estimates. Chi-square tests and Fisher's exact test (where observed number of isolates on sub-culture was less than five) were used to determine whether isolation rates at each culture interval (CI) were significantly different.

Ethical clearance

Permission to conduct the evaluation and ethics approval was granted by the University of the Witwatersrand Human Research Ethics Committee (Certificate No: M160667) as part of the GERMS-SA Surveillance programme for sexually transmitted infections (STIs).

Results

Recruitment and specimen collection occurred over a four-month period (26 April 2016 – 26 August 2016). Of sixty-six urethral discharge specimens collected, 62 were culture-positive for *N. gonorrhoeae* on direct specimen inoculation (gold-standard method). Another four specimens were excluded from the study: one had a missing eSwab® at ambient temperature, and daily culture examination was not consistently done over three days for three specimens. Overall, 58 samples were available for complete evaluation.

At 24 h of collection, the culture yield from Copan eSwabs® at both room and fridge temperatures was 100% (58/58; 95% CI: 93.8–100%). Subsequently the yield of *N. gonorrhoeae* from eSwabs® held at room temperature versus refrigerator temperature was as follows: 79.3% vs 87.9% at 48 h; 60.3% vs 67.2% at 72 h; 22.4% vs 60.3% at 96 h; and, 3.4% vs 53.4% at 120 h, respectively (Table 1; Figure 1). Thereafter, the *N. gonorrhoeae* culture yield from Copan eSwabs® stored in the refrigerator declined to 27.6% and 12.1% at 144 and 168 h (i.e.

Table 1: Comparison of culture yield of *Neisseria gonorrhoeae* at various intervals from time of collection on Copan eSwabs® stored at ambient and fridge temperatures

Time from collection	<i>n</i> at 24 h (% 95% CI)	<i>n</i> at 48 h (% 95% CI)	<i>n</i> at 72 h (% 95% CI)	<i>n</i> at 96 h (% 95% CI)	<i>n</i> at 120 h (% 95% CI)	<i>n</i> at 144 h (% 95% CI)	<i>n</i> at 168 h (% 95% CI)
Ambient/room temperature (RT) (<i>n</i> = 58)	58 100 (93.8– 100)	46 79.3 (67.2– 87.7)	35 60.3 (47.5– 71.9)	13 22.4 (13.6– 34.7)	2 3.4 (0.9– 11.7)	0 0 (0– 0.6)	0 0 (0– 0.6)
Fridge temperature (FT) (<i>n</i> = 58)	58 100 (93.8– 100)	51 87.9 (77.1– 94.0)	39 67.2 (54.4– 79.9)	35 60.3 (47.5– 71.9)	31 53.4 (40.8– 65.7)	16 27.6 (17.8– 40.2)	7 12.1 (6– 22.9)
<i>p</i> -value (RT = FT)	1.00	0.210	0.410	<0.001	<0.001*	<0.001*	<0.001*

*Fisher's exact test.

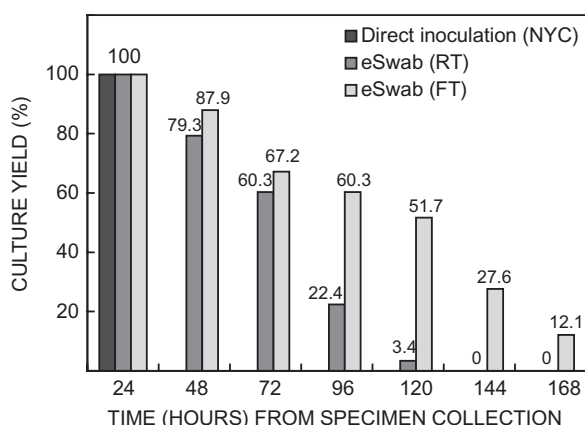


Figure 1: Comparison of *Neisseria gonorrhoeae* culture yield from Copan eSwabs® stored at room and refrigerator temperatures at 24 h time intervals.

days 6 and 7), respectively; however, there was no bacterial growth from eSwabs® held at room temperature at similar time points from collection. From 96 h (day 4 of collection) onwards, the yield from eSwabs® held at refrigerator temperature showed significantly higher isolation rates compared to those maintained at room temperature ($p < 0.001$).

Discussion

N. gonorrhoeae is a fastidious bacterium that autolyses rapidly. Culture recovery of *N. gonorrhoeae* remains important for the diagnosis of gonorrhoea, and is essential for surveillance of antimicrobial susceptibility and monitoring resistance trends.

Ideally, clinical specimens should be directly inoculated onto nutritive, selective (antibiotic-containing) medium such as New York City agar and immediately incubated at 37°C in 5 to 10% CO₂.⁷⁻⁹ However, this is impractical when laboratories are not situated in close proximity to clinical areas as the requisite facilities are not available in peripheral healthcare clinics or at surveillance sites. Protective swab transport systems have to be used to preserve the organism in clinical specimens during transfer to the laboratory for culturing. These include non-nutrient, semisolid or liquid transport media such as Amies or Stuarts transport medium.^{10,11}

Our study has revealed that *N. gonorrhoeae* is able to maintain 100% viability for 24 h of storage in Copan eSwabs® at both room and refrigerator temperatures, in accordance with manufacturer's claims. Additionally, at days 4 and 5 of collection, approximately 60% and 50% of isolates, respectively, were viable in eSwabs® held at fridge temperature. The viability dropped to unacceptably low levels of 22.4% and 3.4% at room temperature for the same respective time intervals from collection, and from day 4 onwards the culture yield was significantly lower compared to those from refrigerated eSwabs®.

The culture swab is perhaps the most widely used specimen collection device. Micro-organisms do not survive equally well in similar types of swabs from different commercial sources. Major differences were observed in the survival of *N. gonorrhoeae* over a six-hour period in specimens collected using swabs from three different manufacturers and stored at room temperature in modified Stuart's transport medium.¹²

The Copan eSwab system uses a modified Amies transport medium comprising inorganic phosphate buffer, calcium salt, magnesium salt and sodium chloride, with the addition of sodium thioglycollate as a reducing agent.¹³ The tip is flocced with soft nylon fibre which facilitates sample elution into the transport medium on contact. The sample should ideally be transported to the laboratory within 2 h of collection for optimal viability, but the manufacturers state that a processing delay of up to 24 h if stored at room or fridge temperatures is acceptable for *N. gonorrhoeae*.

The utility of Amies transport medium from Diagnostic Media Products, NHLS, in comparison to commercial Trans-isolate medium for Dacron swab-collected endourethral specimens for *N. gonorrhoeae* has previously been evaluated in our laboratory.¹⁴ At 48 h of storage at room temperature, the Amies medium showed superior recovery rates of *N. gonorrhoeae* growth than the Trans-isolate medium, which would enable organisms to survive adverse conditions in the field and during shipment for a longer period.

Charcoal is present in certain transport media to neutralise toxicity and thereby improve culture yield. A comparison of direct inoculation with two commercial swab transport systems, Copan Amies gel agar with and without charcoal, revealed no difference in gonococcal culture yield at 24 h using the swab systems (95% of directly-inoculated specimens).¹⁵

Others have confirmed that refrigeration does not impair recovery of *N. gonorrhoeae* from transport media, and may in fact enhance organism survival during the holding period.¹⁶ It has additionally been postulated that overgrowth of contaminating commensals may be inhibited by refrigeration, contributing to improved gonococcal preservation.

A study comparing two culture transport systems for the maintenance of fastidious aerobic organisms showed that, in general, the recovery of test organisms, including *N. gonorrhoeae*, was better with swabs held at 4°C.¹⁷ Similarly, a comparison of the Copan transystem swab (containing a polyurethane foam sponge soaked in liquid Stuarts medium) and Copan eSwab revealed that the eSwab was significantly better at 4°C, and marginally better at room temperature, than the transystem swab for the recovery of *N. gonorrhoeae* at 24 h.¹⁸

An added advantage of the Copan eSwab system relates to the preservation of nucleic acids of bacteria and viruses.³ A study using eSwabs as a transport system for specimen testing by molecular methods, demonstrated its suitability for the real-time PCR detection of urethral discharge pathogens such as *Chlamydia trachomatis* and *N. gonorrhoeae*.¹⁹

Conclusion

This study confirms that Copan eSwabs® are a suitable transport and maintenance system for the preservation of *N. gonorrhoeae* in clinical urethral discharge specimens. A holding time of 24 h from collection is optimal for organism recovery, but if maintained at temperatures of 2–8°C during transport, a survival rate of at least 50% can be expected at day 5 of collection, making the system an attractive option for transport of swabs from peripheral surveillance sites to a central laboratory.

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